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REMARKS

Claims 1-55 are pending in the present application, with claims 30-55 having been withdrawn from consideration following Applicants' election in response to a restriction requirement. Claims 1 and 2 have been amended herein. Upon entry of the current amendment, claims 1-29 will be under examination.

Regarding the amendments

The specification has been amended herein to correct a typographical error in the paragraph on page 5, lines 10-23, as requested by the Examiner. This amendment to the specification adds no new matter.

Claim 1 has been amended to indicate that a satisfying amino acid is assigned to two or more of the labeled weighted directed edges. This amendment is supported in the specification, for example, on page 52, line 26, to page 53, line 6, which describes that labeled weighted directed edges can be labeled with a satisfying amino acid; and page 17, lines 13-18, which describes a satisfying amino acid as an amino acid having a mass that matches a mass measurement of an amino acid or that matches the difference in mass of two mass signals corresponding to a polypeptide and a fragment thereof that differs in size by a single amino acid.

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Claim 2 has been amended to replace step labels (a) and (b) with step labels (e) and (f). This amendment adds no new matter.

As set forth above, the claim amendments are fully supported by the specification as originally filed and do not introduce new matter. Accordingly, entry of the amendments is respectfully requested.

Regarding the objection of disclosure

The Office Action states that the disclosure is objected to because of a typographical error in the word "polypeptide" on page 5, line 22. Applicants have herein amended the specification to correct this typographical error and accordingly request withdrawal of this objection.

Regarding the rejection under 35 U.S.C. § 112, first paragraph, enablement

The objection to the specification and corresponding rejection of 1-29 under 35 U.S.C. § 112, first paragraph, as allegedly lacking enablement in the specification, are respectfully traversed.

The Office Action states that the specification is enabling for a method of determining an amino acid sequence of a polypeptide using the following eight step procedure:

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- 1) filter low intensity peaks from both input spectra to reduce noise;
- 2) where integer values of n range from 1 to N create a node with mass (m) and number of methyl esters (n) if peaks are with m in the d_0 spectrum and mass $m=n\delta \pm \epsilon$ in the d_3 spectrum;
- 3) assign the created node an intensity value equal to the product of the intensities of the 2 peaks;
- 4) create a single source node with $m=M$ and $n=N$, and a single terminus node with $m=0$ and $n=1$;
- 5) add a labeled weighted directed edge from node 1(m_1, n_1) is added to node 2 (m_2, n_2) if the edges if $m_1=m_2+\text{mass}[\text{methylated amino acid(s)}] \pm \epsilon$ and $n_1=n_2$ or $m_1=m_2 + \text{mass}[\text{aspartic or glutamic acid methyl ester}] \pm \epsilon$ and $n_1=n_2+1$;
- 6) a label, corresponding to the satisfying amino acid(s) and a weight equal to the product of the two node intensities, is assigned to the edge;
- 7) the highest scoring path through the graph from the source node to the terminus node is derived from the score of a path computed as the sum of the weights of its edges;
- 8) the sequence of the polypeptide is given by (from carboxyl to amino-terminus) the labels of the edges of the highest scoring path.

However, the Office Action alleges that the specification fails to describe procedures other than the eight step procedure described above that would have been used by one skilled in the art to determine an amino acid sequence of a polypeptide according to the claimed method. Applicants

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respectfully submit that the specification teaches and provides guidance for practicing the full scope of claim 1, which can be achieved using only a portion of the eight step procedure, as well as using multiple variations of the steps.

Although the particular eight step procedure pointed out in the Office Action describes an embodiment of the claimed method, all of these steps need not be performed to determine an amino acid sequence using the claimed method. For example, step 1 (filter low intensity peaks from both input spectra to reduce noise) is not required for determining an amino acid sequence using the claimed invention. Rather, the specification teaches that this step can be included to enhance accuracy if desired (page 53, lines 26-28).

Similarly, step 7 (the highest scoring path through the graph from the source node to the terminus node is derived from the score of a path computed as the sum of the weights of its edges) is not required for determining an amino acid sequence using the claimed invention. Instead, the specification discloses that this step can be included when it is desired to obtain more extensive amino acid sequence by teaching, for example, that the boundaries of nodes can be utilized by creating a source node with mass M , number of labels N , and fixed intensity I_s , (M, N, I_s) and/or a terminus node with mass 0 , n_0 and fixed intensity I_t , $(0, n_0, I_t)$ (page 54, lines 8-16).

Additionally, step 8 (the sequence of the polypeptide is given by --from carboxyl to amino-terminus-- the labels of the

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edges of the highest scoring path) is not required for determining an amino acid sequence using the claimed invention. In particular, the specification discloses that a polypeptide sequence can be determined either from carboxyl to amino-terminus or the reverse, for example, by teaching that "differential labeling of polypeptides at either the amino- or carboxyl-terminus is advantageous for de novo sequencing methods because the label serves as a reference point and allows the orientation of the polypeptide sequence to be determined (page 35, lines 12-16)." The specification further teaches that a variety of reactive groups that can be used to incorporate a differential label at an amino- and/or carboxyl-terminus or internal site of a polypeptide. Exemplary reactive groups include a reactive group that can react with carboxyl groups (page 30, line 30, to page 31, line 1); a reactive group that can react with amines (page 31, lines 1-4); as well as a reactive group that can react with oxygen, sulfur, phosphate groups, and other moieties (page 31, lines 4-10). Thus, the specification provides enablement for practice of the claimed method by determining an amino acid sequence from amino- to carboxyl-terminal end as well as from carboxyl- to amino-terminal end.

Applicants submit that the specification further provides enablement for the full scope of claim 1, for example, by teaching that a graph from mass spectra of two or more differentially labeled polypeptides can be constructed using data obtained with a variety of labels in addition to methyl esters. Specifically, the specification teaches that polypeptides can be derivatized by alkylation, acylation, carbamylation, iodination,

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and other modifications; that free amino groups can be derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups; that free carboxyl groups can be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides; that free hydroxyl groups can be derivatized to form O-acyl or O-alkyl derivatives; and that imidazole nitrogen of histidine can be derivatized to form N-im-benzylhistidine (page 8, lines 15-28).

In addition, the specification teaches that polypeptides can be labeled with a moiety having a stable isotope, such as isotopically heavy and light versions of hydrogen, carbon, oxygen, nitrogen, sulfur and selenium (page 12, lines 16-30); that polypeptides can be differentially labeled by labeling one polypeptide in a sample by any method and leaving the other polypeptide unlabeled (page 13, lines 1-3); and by labeling one polypeptide with a moiety such as an ICAT[®] reagent and labeling another using a different moiety (page 13, lines 3-9). The specification further teaches specific exemplary linkers useful as differential labels, such as 4,7,10-trioxa-1,13-tridecanediamine based linker and its related deuterated form, 2,2',3,3',11,11',12,12'-octadeutero-4,7,10-trioxa-1,13-tridecanediamine (page 32, lines 25-29).

Moreover, a variety of additional polypeptide modifications useful for differential labeling of a polypeptide from which amino acid sequence is determined using the claimed method were well known to, and routinely performed by, those

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skilled in the art at the time of filing the present application. Applicants submit that because differentially labeled polypeptides can be labeled using a variety of differential labels as described above, mass spectra having a variety of characteristics expressible as a node can be used to construct a graph in the claimed method. In contrast to the eight step procedure set forth in the Office Action, such a graph need not be limited to representing d0 and d3 spectra; nor do mass values need to be limited to methylated amino acid(s), or aspartic or glutamic acid methyl ester masses. In this regard, those skilled in the art would have (a) been able to select from a variety of differential labels in addition to methyl ester; and (b) would have understood that the mass differential of label (δ), number of labels (n) and other parameters can vary depending on the selected label, and therefore would have been able to practice the claimed invention using a variety of differentially labeled polypeptides.

Regarding step (c) of claim 1, Applicants submit that the specification teaches and provides guidance to those skilled in the art for the full scope of the method by describing multiple weight characteristics relevant to "labeled weighted directed edges." In particular, the specification teaches that properties of signals can be combined into weighting characteristics depending, for example, on the type of properties to be combined, and that numbered values, for example, can be added, subtracted, multiplied, or divided (page 15, lines 3-10). Therefore, Applicants submit that the specification provides enablement for practice of the claimed method using procedures in

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addition to "assigning a created node an intensity value equal to the product of the intensities of the two peaks," as is stated in step 3 of the eight step procedure set forth in the Office Action.

In view of the above remarks, Applicants respectfully request that the Examiner reconsider and remove the enablement rejection under the first paragraph of 35 U.S.C. § 112.

Regarding the rejection under 35 U.S.C. § 112, second paragraph

The rejection of claims 1-29 under 35 U.S.C. § 112, second paragraph, as allegedly indefinite is respectfully traversed.

The Office Action alleges that claim 1 is indefinite for failing to recite a final process step that agrees with the preamble. Applicants submit that claim 1 is clear and definite as written. In this regard, one of ordinary skill in the art would have understood that practice of the steps of claim 1 would have lead to determining an amino acid sequence, given that the claim recites adding a labeled weighted directed edge to the graph between any two nodes corresponding to a mass of an amino acid, and accordingly would have understood the metes and bounds of the claim. Nevertheless, Applicants have amended claim 1 to recite a step of assigning a satisfying amino acid to two or more of said labeled weighted directed edges, thereby determining said amino acid sequence. In view of the above, Applicants request

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removal of the rejection of claim 1 and dependent claims 2-29 under 35 U.S.C. § 112, second paragraph.

Claim 2 has been rejected as allegedly indefinite for delineation of the recited steps as (a) and (b). The Office Action alleges that presentation of these steps is confusing as to the order in which they are to be performed.

Applicants submit that claim 2 is clear and definite as written in view of description of the recited steps in the specification. In this regard, the specification teaches that step (a) and (b) as originally set forth in claim 2 can be performed in any order with respect to each other, as well as with respect to steps (a) through (c) as recited in claim 1. Specifically, steps of (a) creating a source node with total mass M , total number of labels N and fixed intensity I_s ; or (b) creating a terminus node with mass 0, minimum number of labels n_0 and fixed intensity I_t , which can be employed to derive information from boundaries of the nodes, can be performed simultaneously with, or at a point after, step (a) of claim 1 (see, for example, page 54, lines 8-12). Nevertheless, Applicants have amended claim 2 to delineate the steps as (d) and (e), rather than (a) and (b), merely to denote that these steps are additional to those recited in claim 1. In view of the above, Applicants submit that claim 2 is clear and definite, and respectfully request removal of this rejection of claim 2 and dependent claims 3 and 4.

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CONCLUSION

In light of the amendments and remarks herein, Applicants submit that the claims are now in condition for allowance and respectfully request a notice to this effect. The Examiner is invited to contact the undersigned agent or Cathryn Campbell with any questions related to this application.

Respectfully submitted,

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Date

Pamela M. Guy
Pamela M. Guy
Registration No. 51,228
Telephone No. (858) 535-9001
Facsimile No. (858) 535-8949

McDERMOTT, WILL & EMERY
4370 La Jolla Village Drive
7th Floor
San Diego, California 92122